RISK ASSESSMENT OF CHEMICAL CARCINOGENS:

IS IT TIME FOR A CHANGE?

By

Robert J. Scheuplein, Ph.D.
Director, Office of Toxicological Sciences
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Washington, D.C. 20204

Thank you, Carol, for the introduction and to ILSI for inviting me to speak to you today.

My subject is the Quantitative Risk Assessment of Carcinogens: Is it time for a change? Before I speculate about the direction in which risk assessment might be headed or ought to be turned, I would like to give you some idea of where I think its come from, what science it rests on, what it is and what it is not.

This will involve a brief scientific history of cancer risk assessment with a few detours to places where it touches social policy or has been influenced by the Congress and the regulatory agencies. This interaction between science and regulation is important to understand, because a part of my thesis will be that despite well over 500 papers on cancer risk assessment, on the bioassay, on cancer thresholds and numerous related subjects, since 1961 (the date of Mantel and Bryan's paper) -- cancer risk assessment is still more of a regulatory tool than a scientific discipline and rests more on regulatory need than scientific plausibility.

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It nonetheless involves several scientific matters and any good history should include them: (Slide 1) - Major Scientific Issues in Cancer Risk Assessment.

These issues are all involved in QRA and the final result - the determination of a safe level - depends on how these issues are resolved. And I'll touch briefly on most of them.

Right after World War I, scientists began to experiment with large colonies of rodents. Shortly thereafter, Yamagiwa and Ichikawa discovered that skin cancers could be induced in mice by the repeated application of gas tars (ref.). The number of chemical agents tested grew steadily from year to year and it became difficult to analyze the available experimental results because of the variety of different methods adopted. By 1930, efforts began at standardizing these methods. (Slide 2) - Paper Title by Twort and Twort.

By 1939, methods were sufficiently well developed that lists of substances with relative potencies were published based on the ability of a compound's capacity to produce tumors in the shortest possible time. (Slide 3) - List of Carcinogenic Compounds Arranged in Descending Order of Potency - John Iball (1939). In this list by John Iball in 1939, the index of potency is the percentage of tumours A divided by the mean latent period B, recorded in the last column.

There were good social reasons for these academic efforts. It was becoming clear that environmental and occupational exposure to

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carcinogenic chemicals were contributing to the world-wide diversity in cancer incidence. Percival Pott had established the connection between soot and scrotal cancer in chimney sweepers several generations earlier. By the 20's it was clear that polycyclic hydrocarbons were the carcinogenic ingredients in soot, tar and oil. Some cancers on the abdomen could be attributed to carrying a basket of live coals beneath the clothes to keep warm in winter; some cancers in the buccal cavity could be attributed to chewing various mixtures of betel, tobacco and lime and some on the palate to smoking cigars. As Richard Doll (1977) has pointed out, oncologists who worked chiefly in Europe and North America tended to regard these incidents as oddities and irrelevant to the production of ordinary cancers. So it took a while for people to associate the majority of cancers with environmental factors, but soon the association became obvious.

These concerns in the 30's and 40's motivated an effort to bring the known occupational hazards under control, either by banning their production or controlling the manufacturing process to reduce exposure to employees. (Slide 4) - Occupational Cancers - Doll (1977). But these occupational hazards could not be responsible for the large observed incidence of cancer. Whole populations, however, had been exposed to lower levels of these same agents. These included polycyclic hydrocarbons, produced by the combustion of coal, wood and oil. It was known, for example, that residents of large towns in the U.K. may have been exposed - mainly through the combustion of domestic coal - to something like 1/100 the amount of benzo(a)pyrene regularly inhaled by men working in the manufacture of coal gas and these men experienced only

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an 80% excess risk of lung cancer (Doll, et al, 1972). It was easy enough to dismiss the corresponding risks on the grounds that the doses were minute, but one did not then (or now) assume for cancer the existence of a threshold. So some form of quantitative relationship between the dose and the resulting incidence was needed. But in the absence of such a relationship, decisions had to be made and FDA banned carcinogens from food during the years well prior to the enactment of the Delaney Clause. Arnold Lehman, the chief toxicologist at FDA in the 40's, stated in an article in 1949 that:

> "a finding that a substance caused cancer in animals was regarded as so 'alarming' as to exclude it from consideration."

In 1945, the FDA banned Butter Yellow; in 1950 Dulcin and P-4000, two artificial sweeteners; in 1950 also tonka beans and coumarin; and in 1959, aminotriazol on cranberries, all on the grounds they were carcinogens and had no place in foods.

The reasons that scientists were unwilling to assume the existence of thresholds for carcinogens are interesting - primarily because they were largely theoretical. Essentially the argument recast in modern terms went like this: Cancer is caused by agents known to be mutagenic suggesting that at least one crucial, rate limiting step is a somatic mutation. This focused attention on the nature of the genes that undergo mutation and on the amount of chemical needed to affect that change. It was argued that only one molecule was necessary to produce a mutation in the DNA within the nucleus of a cell. This in turn could lead to a

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miscoding sufficient to produce eventually a malignant cell. The cell then can reproduce itself in an irreversible and unregulated manner to yield a malignant tumor.

But this is theory! What does the experimental evidence show? So far, for any carcinogenic or mutagenic response in any given situation, be it man, mouse, isolated organ or a Salmonella plate assay, there is a demonstrable threshold or "no effect level." In thousands of studies with hundreds of thousands of animals, not a single carcinogen has been found that has not exhibited an experimental threshold. However, animal studies are insensitive and thresholds will vary from individual to individual. It is completely impractical to determine the level at which the most susceptible individual in the whole population might fail to respond. And worse yet, if a gargantuan animal study were done, assuming all the experimental difficulties involved in such a study could be overcome, people would point out that experimental animals are more inbred than people and the result would probably be discounted.

The one-molecule theory has to be argued at the theoretical level, so let's look at it. Nothing (in the one-molecule theory) is mentioned about the relationship between the intake dose and the final concentration of the chemical carcinogen in the nucleus of the cell where it interacts with DNA. Substances that are ingested have to be absorbed, distributed and metabolized usually before they can reach critical organs in chemically activated form. Then the activated molecules have to run a gauntlet of sequestration by other uninvolved macromolecules and overcome

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diffusional barriers before they can first enter a cell, and then later enter the nucleus of a cell.

When the carcinogen is in the nucleus and poised to react with DNA, nothing is mentioned (in the one-molecule theory) about the constraints imposed on chemical reactions by the requirements of mass action or the need to acquire a transition state configuration or an activation energy prior to reaction, or even that the final adduct be somewhat stable so that it can last long enough for replication.

And if a reaction with DNA does occur, nothing is mentioned about the intron content of the DNA, that the amount of DNA in the nucleus that is not overtly expressed as protein may amount up to 90% of the total. These regions of DNA, which correspond mostly to the centromeric parts of the chromosomes almost certainly instruct no other process than their own replication. And finally nothing is said of the ability of the organism to accommodate to adverse effects - in this case by DNA repair mechanisms and enzyme induction. An example on this last point was published in 1977 by Tony Pegg of the Hershey Medical Center.

Dimethylnitrosamine (DMN) is a potent carcinogen in many species. It is well established that DMN exerts its carcinogenic effect after its metabolic conversion into a reactive methylating agent. The electrophile then methylates DNA nucleosides which are likely to miscode. One particular adduct - O^6 - methylguanine appears to be promutagenic or tumorigenic in several studies and it has been identified as probably the adduct responsible for tumor induction in animals.

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What Pegg did was to give DMN to rats and then analyze the livers for the 0^6-adduct after 4 and 24 hours. His results are shown in the slide. (Slide 5). 0^6- Methylguanine Levels in Rat Liver DNA At first glance, there appears to be a linear relationship between the administered dose and the formed adduct. But if the data are extended to lower doses, this is shown not to be true. At very low doses, 0^6-adducts levels are many times less than expected on the basis of linearity. Later studies showed that a saturable enzymic repair system was responsible for the removal of the 0^6-adduct and that the repair system operated in both liver and kidney cells.

These studies don't necessarily imply an absolute threshold for tumor induction for DMN because it was possible to detect some 0^6 - adduct in DNA 24 hours after a single dose and we don't know what level of 0^6-methylguanine may be necessary to initiate tumor induction. But they do show that liver and kidney can protect itself against a low-dose, carcinogenic stimulus and that linear extrapolation is probably unjustified at low doses. These studies by Pegg are pertinent because they provide information well below the observable tumorigenicity range - over a 20,000 fold dose range - and measure the concentration of the specific adduct identified to be the one likely to initiate tumors.

In summary, there are many reasons to contest the "one-molecule theory" and to anticipate, in conformity with the animal evidence, that significant concentrations of a carcinogen might be required to elicit cancer. But nevertheless, the "one-molecule" concept or its equivalent that exceedingly small levels of carcinogens ingested daily for a lifetime

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could be harmful, prevailed - in the 40's and 50's, and I suspect prevail today. Now the question of the existence of a threshold in an individual is a problem in biology - the question of determining the range of possible susceptibility in a large population that is assumed to have individuals capable of responding is a problem in the statistics of sampling to which we now turn.

We have to skip over (in the interests of time) a rich part of the biological science that was developed in the 40's and 50's to arrive at our principal focus, the idea of using risk assessment for setting safe population exposure levels for carcinogens, This idea was first published, so far as I am aware, by Mantel and Bryan in 1961.

They showed that a negative animal study - particularly a small one with 100 animals or so, does not necessarily demonstrate that the treatment was safe. Studies of feasible size can be used to establish directly only risks of the order of 1/100 or higher. When a study with 100 animals is negative, we can only claim that we are reasonably sure (assurance level of 99%) that the true risk is no greater than 4.5 percent. (Slide 6) - Interpretation of Negative Studies. Mantel and Bryan then proposed to rely on the dose-response principle and extrapolate from this upper limit conservatively to human exposure levels. They examined the dose-responses of many chemicals and concluded that most chemical responses involving lethality decreased very rapidly, with dose-response slopes steeper than 10 or more probits per ten-fold dose dilution. They noted probit slopes for the therapeutic effects of antibiotics of 2-3, and still lower probit slopes, the order of 2, in

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virus assay work. From this experience they felt that a probit slope of one per ten-fold dose dilution would likely be quite conservative for carcinogens. This would mean in the example above that the safe dose corresponding to a risk of 1/100 million would be 1/8,300th of that which produced no tumors in the actual study.

This 8,300 corresponds to a safety factor based on an upper confidence limit to a negative study. Later Carrol Weil would propose a factor of 5000 for known carcinogens, using a factor of 10 for animal variation, a factor of 10 to translate animal results to human, a factor of 10 for cancer on the theory that it is less reversible than other toxic effects and a factor of 5 if the data used were a minimum effect level. (Weil 1972).

The Delaney Clause had been passed by Congress a few years earlier as part of the Food Additives Amendment in 1958. In 1960, there were hearings in the House to deal with some unfinished business regarding Color Additives. Cancer experts testified to the Committee that there was a great deal of uncertainty about cancer induction at low doses. A report prepared by G. Burroughs Mider, then Associate Director for Research at NCI, was quoted by the Secretary of HEW at the hearings. It had an impact on the Committee. It stated:

> "No one at this time can tell how much or how little of a carcinogen would be required to produce cancer, or how long it would take the cancer to develop."

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The Secretary also said,

> "Whenever a sound scientific basis is developed for the establishment of tolerances for carcinogens, we will request the Congress to give us that authority."

and also,

> "So long as the outstanding experts in the National Cancer Institute and the Food and Drug Administration tell us that they do not know how to establish with any assurance at all a safe dose in man's food for a cancer-producing substance, the principal in the anticancer clause is sound."

The Congressional response was, of course, predictable and the new Color Additives Amendments of 1960 contained its own Delaney anticancer clause. Congress concluded that there was too much uncertainty and it would require FDA to regulate on the side of caution by banning all animal carcinogens from the food supply. Of course, it was only 1960 - analytical methods were typically capable of detecting a few parts per million at best. There were relatively few carcinogens known and it was not appreciated that traditional food and spices and ordinary cooking practices would eventually be found to account for many if not most of them. The widespread contamination of food by low levels of environmental contaminants like dioxins, PCBs and aflatoxin had not yet been discovered. Nor was the fact that the failure to specify "safe" levels would assure the triggering of the Delaney Clause on food and color

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additives as methods improved to the point of being capable of detecting trace carcinogenic contaminants.

This was the scientific and regulatory setting when in 1969 the FDA gathered together a group of scientists to consider how food additives and pesticides should be tested and evaluated for possible carcinogenicity. The Report of that FDA Advisory Committee on Protocols for Safety Evaluation and in particular the Panel on Carcinogenesis was published in 1971.

This report stated among other things that:

1) Testing should be done at doses and under experimental conditions likely to yield maximum tumor incidence;

2) And that at least two species should be used for all carcinogenicity tests, and that,

3) For compounds judged carcinogenic at test levels, a virtually safe dose could, in principle, be estimated by downward extrapolation using some arbitrarily selected but conservative dose-response curve.

These recommendations initiated the regulatory use of the MTD bioassay and linear risk assessment. Details on both the bioassay and the method of extrapolation would evolve, but this 1971 Panel Report gave their

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development official sanction. The Panel's recommendations were motivated essentially by two concerns -

1) the fears over persistent low doses of carcinogens in food

2) the limitations of negative studies on small numbers of animals

It was clear that no unqualified negative answer is ever possible. That all a negative study can do is to supply an upper limit to the possible carcinogenicity. It was pointed out that these upper limits are uncomfortably large. Even with as many as 1000 test animals and using only 90% confidence limits, the upper limit yielded by a negative experiment is 2.3 cancers per 1000 test animals.

The report contains the following statement:

> "No one would wish to introduce an agent into a human population for which no more could be said that it would probably produce no more than 2 tumors per 1000."

So how does one increase the sensitivity of the bioassay with a limited number of animals? The answer - increase the dose well beyond the anticipated use level and extrapolate the results down to these low doses. If the study is positive, the fidelity of the extrapolation depends on the dose-response curve - a small part of which is accessible in the experimental range. And if the study is negative, it has a theoretical positive upper bound.

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Mantel and Bryan had already introduced the concept of using this upper limit of the negative study to base the downward extrapolation on, using a 1 probit per 10 fold dilution slope. And so, in principle, quantitative extrapolation as a regulatory method (QRA) was approved.

But it was never assumed that cancer necessarily occurred at low doses, but only that if it did, it would be safely bounded by the extrapolation procedure. Now extrapolating a positive response instead of an upper limit to a negative response makes no essential difference as Starr and others have shown unless we know what the dose response curve is. Since our extrapolation models are quite simplistic and without an adequate biological basis - there need not be any cancer at all at low doses - and if there is, we have no idea about its actual dose response. The notion that one can calculate expected values of an actual risk from such an analysis is really quite bizarre. Extrapolation using bioassay data 3-4 orders of magnitude removed is not a procedure for estimating risk - it's regulatory standard setting - It's not science, it's policy. And it's conservative or, if you will, prudent policy - and I'm just describing it, not advocating it.

The scientists who wrote the 1971 Protocols Report had to face two problems. The QRA procedure they were recommending was extremely conservative, it would ban any carcinogenic food additive, because the amount of additive is usually substantial. Remember the additive has to be used in amounts sufficient to accomplish its intended effect. But this didn't really bother anyone at the time - carcinogenic additives had no place in food anyway. And they pointed out that this application of

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QRA would be consistent with the Delaney Clause. They didn't think back then in terms of impurities and low level environmental contaminants - and if they had, I don't think they would have recommended high to low dose extrapolation. I believe this is true because of how they handled the second problem.

If you tested a food additive in a carcinogen bioassay and the result was negative, the logical result of their analysis would require a downward extrapolation from the upper 90% confidence limit ala Mantel and Bryan. But again, because of the substantial amount of food additive required for a functional effect - typically at least several ppm, this extrapolation would ordinarily result in the ban of the additive at effective and useful doses. What did they do?

They ignored their discussion on QRA, they ignored Mantel and Bryan and said that the sensible thing to do was to use a 100-fold safety factor! Their statement was that for agents not judged carcinogenic the use of QRA to estimate a safe dose would be logical, but would give a level so low as virtually to exclude from use agents for which there was no positive evidence of carcinogenicity. And they wouldn't do it.

This commonsense approach to the cancer problem was soon to be challenged by two related difficulties that defied easy solution. The first was how to deal with animal drug residues - those in food-producing animals as the result of ingestion of added drugs for prophylaxis, for the treatment of disease or for growth promotion. In 1962, Congress had put yet another Delaney Clause in the Act with the Animal Drug Amendments - this

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time though with a legal loophole called the DES Proviso. It said in effect that you could use effective animal drugs even if they were carcinogenic so long as none remained in the edible tissue of the animal after slaughter - no residue would be permitted and the FDA was given the task of approving the analytical method to assure it. This ushered in the era that some industry groups characterized as "chasing zero."

The other related difficulty occurred in food and color additives. Analytical methods were becoming so sensitive that traces of carcinogenic contaminants were being found, particularly in colors. It was hard not to find carcinogenic derivatives of aniline, a carcinogen, in aniline-based colors. And so the question was how do you regulate a substance which does not test out as a carcinogen itself, but which contains a chemical at low but detectable levels which is a known carcinogen? Both of these problems would challenge FDA for many years, the first culminating in the final SOM document in 1985, the other in the constituents policy in 1983. The need for both a procedure for risk assessment and a level of acceptable risk were common to both issues and of course they are interrelated. If you use the Probit model and a 10^{-8} acceptable risk level, you come out about the same place as if you used a linear model and a 10^{-6} acceptable risk level.

Extrapolation Models and Background Additivity

In the late 70's and early 80's, there was a good deal of debate over the best form of the extrapolation model. The original Probit model of Mantel and Bryan was considered too arbitrary and not conservative enough - with the developing trend toward a 10^{-6} or one in a million

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acceptable risk level. The One-hit model came from the one molecule theory and was easier to explain, but it wasn't a good fit for most data. There were other models. You may recall the Logit, the Weibull, the Multihit and the Gamma Multi Hit - all of them competing with the Multi-Stage. None of these were based on biology. The critical steps and mechanisms in the development of tumours were and are still unknown. But the multistage model had the best biological credentials, having first been used to explain the steep increase in the age adjusted rates of some cancers in humans by Armitage and Doll in 1961. And most people believed cancer was a multistage process, so there was a simulacrum of a biological basis. Since the early 80's, the strongly curvilinear models have virtually been abandoned. What was that? Well, first the low level risks that emerged from these different models were embarrassingly divergent. When the various models were applied to the risk of saccharin by the NAS in 1978, the risk estimates ranged over 5 million.

(Slides 7 and 8) - Saccharin Risk Estimates (1978).

If OMB had been paying attention back then, they would have been exultant - this risk assessment certainly made the uncertainties in modelling crystal clear! Today our risk assessments don't differ very much. EPA ordinarily uses the Multistage with an algorithm that constrains it to be linear and FDA uses the Gaylor-Kodell procedure for most carcinogens, which is designed to be linear. The other models could not be easily linearized and were abandoned. Since then our risk assessments have been more nearly in agreement, more uniformly conservative and much less revealing of still unresolved uncertainties. The linear multistage

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yielded greater conservatism and had the right name, but what really clinched linear risk assessment was an idea published in 2 or 3 papers in 1977 and 1978 by Crump, Hoel, Peto and their co-workers. These papers contained a notion which today is unfortunately part of quantitative risk assessment mythology, namely that there are sound biological reasons for believing that every carcinogen response curve is linear at low dose rates, as far as humans are concerned. This proposition rests on the presence of background carcinogens and the way they interact with the carcinogen in question. The reasoning is part biological and part mathematical.

The biological part

Approximately one of five Americans develops a cancer, and every person is exposed to thousands of carcinogens in food, in the environment and even endogenously. This 20% background rate, from these many different chemicals must surely provide some significant mechanisms that are shared by the carcinogen in question. In other words, the carcinogen being added and some of the background carcinogens must share a common pathway to carcinogenesis - and thus produce cancer through an identical mechanism. Their effects are functionally indistinguishable.

Now the mathematical part

As the slide shows, (Slide 9) - Background Additivity, the cancer incidence I(d) will be a linear function of the dose rate d at low dose rates provided that the slope $\left(F^{1}(D_{o})\right)$ is positive. They defend the assumption that the slope is positive by arguing that even if there were a threshold, it would be a threshold for each cell; there would be a

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distribution of these and at least one of which would be below the critical value. Since cancer is believed to be of single cell origin, this one activated cell would initiate the cancer, $F^1(D_o)$ would be positive and the probability of response would be linear at low doses. They conclude

"... in environments already containing appreciable amounts of carcinogenic processes, the effects of any slight addition to these processes will be proportional to the amount added. ... its implications are that much previous investigation of the form of the dose-response relationship at infinitesimal doses is irrelevant to the interpretation of animal studies for the formulation of social policy."

It's hard to know what to say in the face of such confidence - for which there is no experimental data at all. These excathedra pronouncements are not believed by everyone but they continue to haunt some people including some in the regulatory agencies. The implications are, if this is true, that dose-response curves become approximately linear just below the observable range - so long as they are roughly linear in the observable range. Not everyone believes this - I certainly don't. Alice Whittemore, Mel Andersen and other pharmacokineticists still believe that tumor probabilities are proportional to effective doses and these generate very non-linear dose-response curves. And I suppose that the folks in Kurt Harris's lab at NCI still feel they have accomplished something by finding mutations in p53, a putative tumor suppressor gene in human hepatocellular carcinomas in China, despite the theory that says

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these mutations are expected to be produced by "background carcinogens," not just aflatoxin.

MTD

The use of MTDs (Maximum Tolerated Doses) had been challenged throughout the period. Perry Gehring and Phil Watanabe had shown in 1976 that large doses could exceed metabolic and physiological thresholds, leading to prolonged retention in the body, formation of different metabolites and in some cases disproportionate increases in reactions between reactive electrophilic metabolites and macromolecules. They concluded that dose-dependent alterations in the fate of chemicals must be considered or at high doses you risk the likelihood of disproportionate increases in toxicity including carcinogenesis. They reported evidence of possible dose-dependent effects in styrene, ethylene glycol, aniline, carbon disulfide, 2-naphthylamine, benzopyrene, bis-hydroxycoumarin, salicylamide, amphetamine and sulfobromophthalin (Gehring, et al) (1976).

By the late 1970's, enough bioassay data had accumulated largely owing to NCI and later NTP studies, to provide a sufficient basis to examine the results of the studies for correlations between the responses in rats and mice. In 1979, Crouch and Wilson examined the carcinogenic potencies for 70 chemicals in the two species. They demonstrated empirically that good correlations existed for the potencies between the different species. This was an important finding, because if there were good interspecies correlations between potency estimates for rats and mice, then it was reasonable to believe that humans and animals might also be similar in their carcinogenic responses. But in 1985, Berstein, et al, reported

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that the MTD's used in 186 NCI experiments were also highly correlated with potency.

(Slide 10) - MTD - Potency Correlation.

Correlations between MTDs in rats and mice are not surprising because both species could respond similarly to high doses of different chemicals. However, the strong correlation between these MTDs and the derived carcinogenic potencies is startling. The correlation is surprising because MTDs are determined in a 90 day study and this time period has been regarded as too small a fraction of a rodent's lifetime to reflect the presence of a carcinogenic process - much less predict the strength of the carcinogen. Berstein and co-workers showed (Slide 11) that potency estimates from NCI Bioassays were restricted to an approximately 30-fold range surrounding $\frac{\ln(2)}{TD_{50}}$. The TD_{50} is virtually the same as the MTD. They used a one-hit model to show this and an idealized 2-Group experimental design, but they and others have shown that this high correlation is not sensitive to "reasonable" departures from either the experimental design or the extrapolation model used.

Rieth and Starr (1989), and others since, have investigated these correlations in detail. It's clear now that:

o - The correlations between the MTDs and the estimated potencies are real. They do not depend on a "selected" data base.

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- The correlation in the carcinogenic potency estimates in both rats and mice are determined nearly entirely by the magnitude of the MTD used and only minimally by the extent of the carcinogenic response.

- Based on upper limits, inferred potencies from some substances giving no response in the MTD-bioassay appear to pose a possible carcinogen risk as high as 10,000 times greater than other demonstrated carcinogens.

- There is no reason to believe that the inverse MTD or its equivalent $1/TD_{50}$ should be regarded as a valid indicator of the low dose risk either to animals or to humans.

(Slide 12) - Starr's Comparison of 1/MTD with Potency Estimates of 83 Rat Carcinogens

There is a plausible explanation for the strong 1/MTD vs. potency correlation despite the fact that it is hard to prove. Many believe, as I do, that the high doses used in the bioassays are capable of producing carcinogenic responses not necessarily present at lower doses of the same chemical. Depending on the chemical the mechanisms will vary, e.g., altered metabolic pathways, ala Perry Gehring; altered physiology, e.g., d-limonene, NTA, saccharin, enhanced cell proliferation, ala Bruce Ames; altered endocrine or hormonal status, e.g., mammary and thyroid cancers and many others. If this is true, and more evidence is accumulating that it is, then high-to-low-dose extrapolation of carcinogenic effects, on

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the basis of bioassay results only, is not credible. This is not to say that all high dose carcinogens are not carcinogenic at low doses. But the current NTP cancer bioassay data base with approximately 50% of the compounds testing positive contains many compounds that are probably not carcinogenic at low doses mixed in with many that are. The point is the test does not discriminate between them.

Back to Druckrey

While Secretary Fleming was reading the MIDER Report to Congress in 1960, Hans Druckrey in Germany was preparing to publish a review of his life-long work on chemical carcinogenesis. He published it in 1966 in a review article entitled:

"Quantitative Aspects in Chemical Carcinogenesis."

He and his colleagues were very well recognized - Druckrey, Preusman, Schmahl, Nakayima and others were major contributors to the field of chemical carcinogenesis. Their work spanned 25 years and included the testing of over 100 different chemical compounds in some 10,000 rats.

An example of his work on diethylnitrosamine is shown on the next slide. He administered daily doses in the drinking water until 50% of the animals in each group had liver tumors. The slide (Slide 13) shows that at the lowest daily dose rate (0.075 mg/kg/d) the cumulative dose required to produce cancer in 50% of the animals was only 1/15 of that required at a daily dose of 14.2 mg/kg/d.

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This fact, that lower doses applied for a longer period could be more potent than larger doses of less duration was avidly learned and made a permanent feature of the lore of chemical carcinogenesis. But part of Druckrey's work seems to have been ignored. First, the price that is paid for a more efficient dose response is a longer induction time. And second, the cumulative doses involved are large, comparable to ingesting 1/10 of the MTD daily for a lifetime. This nitrosamine work was published in 1963 and, before that in 1961, Shubik had shown that not only were tumors generally slower developing at low doses, they also were more benign.

The Druckrey data are plotted in the next slide (Slide 14). You can see the increase in the induction time with decreasing dosage. The data show very clearly that 0.075 mg/kg/d is close to a practical threshold based on the fact that the induction time required for the development of the tumors approaches the lifetime of the animals. Druckrey's rats didn't live much longer than 2½ years or $\approx$ 900 days -- and these days they don't live nearly as long. And he concluded that

> "With very low dosage the induction time can be longer than the life expectancy and that this is apparently a limiting factor in carcinogenesis."

He even had some regulatory advice for us:

> "As a basis for future discussions it is proposed, that 1 per cent of the lowest dosage, which, given daily over the whole life span to susceptible experimental animals, produces cancer only at the end of

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the life span, can be considered as the maximum tolerable dose for human beings. This, however, only in such cases, in which a complete exclusion from [the] human is not feasible."

Some Conclusions

I don't have the answers to these current scientific issues in risk assessment, but I do have some suggestions as to how we should behave about them.

CANCER IS A VITAL HEALTH ISSUE - (Slide 15) - AND WE SHOULD TREAT IT SERIOUSLY AND DETERMINE WHERE THE REAL RISKS ARE.

o - Face up to the fact that, as we use Quantitative Risk Assessment today, it is justified almost entirely as a very prudent regulatory standard - if that's what we really want. It does not estimate risk and we will have to expect that it won't for decades.

o - Stop the codification of risk assessment "acceptable levels" and risk assessment methodology in Federal Statutes. We are just creating other kinds of Delaney Clauses.

o - Try harder to examine some of the cancer mythology that underlies our beliefs concerning thresholds, additivity and standard testing procedures for carcinogens.

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o - Try to discourage media hype. Incessant coverage of the risk of real or suspect carcinogens - buoyed up by the exaggerated claims of QRA determined risks - makes it unnecessarily difficult to get the public to appreciate the overwhelming importance of smoking and the diet to cancer causation.

o - Try to discourage the use of health warnings on trivial risks. It was absolutely appalling that for many years the health warning on saccharin in the U.S. was at least as strong as that on cigarettes. THAT'S NOT RISK COMMUNICATION! Cigarettes probably contribute some 150,000 deaths from cancer each year. Saccharin was banned by the FDA in 1977 on the grounds that it wasn't shown to be safe and on the Delaney Clause - not because it was known to produce cancer in humans.

o - Finally, do some good research mechanistic work on cancer. There are, I think, three areas to focus on:

1) Theoretical work

2) Work at the cellular level, biochemistry, oncogenes

3) Work in whole animals - (not MTD Testing)

i. dosing regimens, effects of diet

ii. effective dose studies and pkBP

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iii. mechanism studies in vivo, e.g., foci development

iv. secondary mechanism for non-mutagenic carcinogens

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SLIDES.

BASIC SCIENTIFIC ISSUES IN CANCER RISK ASSESSMENT

- ——— SIZE OF HUMAN CANCER RISK FROM LOW DOSE EXPOSURES
- ——— RELIABILITY OF ANIMAL TO MAN EXTRAPOLATION
- ——— EXISTANCE OF DOSE-RESPONSE FOR CARCINOGENS
- ——— GRADABLE POTENCIES FOR CARCINOGENS
- ——— INSENSITIVITY OF ANIMAL STUDIES
- ——— EXISTANCE AND MEASURABILITY OF THRESHOLDS
- ——— INTERPRETATION OF NEGATIVE ANIMAL STUDIES
- ——— HIGH TO LOW DOSE EXTRAPOLATION
- ——— INDEPENDENCE OR ADDITIVITY OF BACKGROUND

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THE AMERICAN JOURNAL OF CANCER

A Continuation of The Journal of Cancer Research

VOLUME XVII FEBRUARY, 1933 NUMBER 2

SUGGESTED METHODS FOR THE STANDARDISATION OF THE CARCINOGENIC ACTIVITY OF DIFFERENT AGENTS FOR THE SKIN OF MICE

C. C. TWORT AND J. M. TWORT

(From the Laboratories of the Manchester Committee on Cancer)

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IBALL (1939)

TABLE I: *Carcinogenic Compounds Arranged in Descending Order of Potency*

Compound	No. of mice alive when first tumour appears	No. of tumours	Percentage of tumours (A)	Papilloma	Epithelioma	Average latent period (B)	Index (A/B × 100)
1. 9 : 10-Dimethyl-1 : 2-benzanthracene	20	13	65	6	7	43	151
2. Methylcholanthrene (a)	18	18	100	1	17	99	101
3. Methylcholanthrene (b)	8	5	62.5	0	5	151	41
4. Methylcholanthrene (a and b added together)	26	23	88.5	1	22	109	80
5. 3 : 4-Benzpyrene (from pitch)	10	10	100	2	8	127	79
6. 3 : 4-Benzpyrene (synthetic)	9	7	78	2	5	109	72
7. 3 : 4-Benzpyrene (5 and 6 added together)	19	17	89.5	4	13	119	75
8. Cholanthrene	49	28	57	5	23	112	51
9. 5 : 6-*cyclo*Penteno-1 : 2-benzanthracene	14	13	93	1	12	194	48
10. 2-Methyl-3 : 4-benzphenanthrene	16	12	75	5	7	155	48
11. 10-Methyl-1 : 2-benzanthracene	18	12	66.5	2	10	147	45
12. 5 : 6-Dimethyl-1 : 2-benzanthracene	19	16	84	0	16	220	38
13. 6-*iso*Propyl-1 : 2-benzanthracene	15	11	73.5	1	10	204	36
14. 3 : 4 : 5 : 6-Dibenzcarbazole	19	9	47.5	4	5	143	33
15. 3 : 4 : 8 : 9-Dibenzpyrene	17	10	59	0	10	205	29
16. 5-Methyl-1 : 2-benzanthracene	8	7	87.5	2	5	317	28
17. 5-Ethyl-1 : 2-benzanthracene	9	7	77.5	2	5	285	27
18. 1 : 2 : 5 : 6-Dibenzanthracene	65	41	63	8	33	239	26
19. 3 : 4-Benzphenanthrene	18	12	67	5	7	387	17
20. 1 : 2 : 5 : 6-Dibenzcarbazole	9	4	44.5	1	3	263	17
21. 5-*n*-Propyl-1 : 2-benzanthracene	20	6	30	3	3	192	16
22. 3: 4 : 5 : 6-Dibenzacridine	28	11	39.3	2	9	357	11
23. 3'-Methyl-1 : 2 : 5 : 6-dibenzanthracene	25	7	28	1	6	325	9
24. 1 : 2 : 5 : 6-Dibenzacridine	25	6	24	2	4	350	7
TOTALS		305		60	245		

is still the possibility of an error due to the fact that a number of animals may die soon after the first tumours are seen and before the majority have appeared (3) Theoretically it would be an advantage in obtaining a quantitative comparison of the potency of several compounds if all the experiments were carried out on pure-line mice under as nearly as possible the same conditions. This would reduce the variation between the batches of animals used for different compounds, but the results of the comparison would then apply only to that

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FROM DOLL (1977) ORIGINS OF HUMAN CANCER COLD SPRING HARBOR VOL 4

Table 2
Occupational Cancers

Agent	*Occupation*	*Site of cancer*
Ionizing radiations		
radon	certain underground miners (uranium, fluorspar, hematite)	bronchus
X rays, radium	radiologists, radiographers	skin
radium	luminous dial painters	bone
Ultraviolet light	farmers, sailors	skin
Polycyclic hydrocarbons in soot, tar, oil	chimney sweepers	scrotum
	manufacturers of coal gas	skin
	many other groups of exposed industrial workers	bronchus
2-Naphthylamine; 1-naphthylamine	chemical workers; rubber workers; manufacturers of coal gas	bladder
Benzidine; 4-aminobiphenyl	chemical workers	bladder
Asbestos	asbestos workers; shipyard and insulation workers	bronchus pleura and peritoneum
Arsenic	sheep dip manufacturers; gold miners; some vineyard workers and ore smelters	skin and bronchus
Bis (chloromethyl) ether	makers of ion-exchange resins	bronchus
Benzene	workers with glues, varnishes, etc.	marrow (leukemia)
Mustard gas	poison gas makers	bronchus; larynx; nasal sinuses
Vinyl chloride	PVC manufacturers	liver (angiosarcoma)
(Chrome ores)	chromate manufacturers	bronchus
(Nickel ore)	nickel refiners	bronchus; nasal sinuses
(Isopropyl oil)	isopropylene manufacturers	nasal sinuses
*	hardwood furniture makers	nasal sinuses
*	leather workers	nasal sinuses

* Specific agent not identified.

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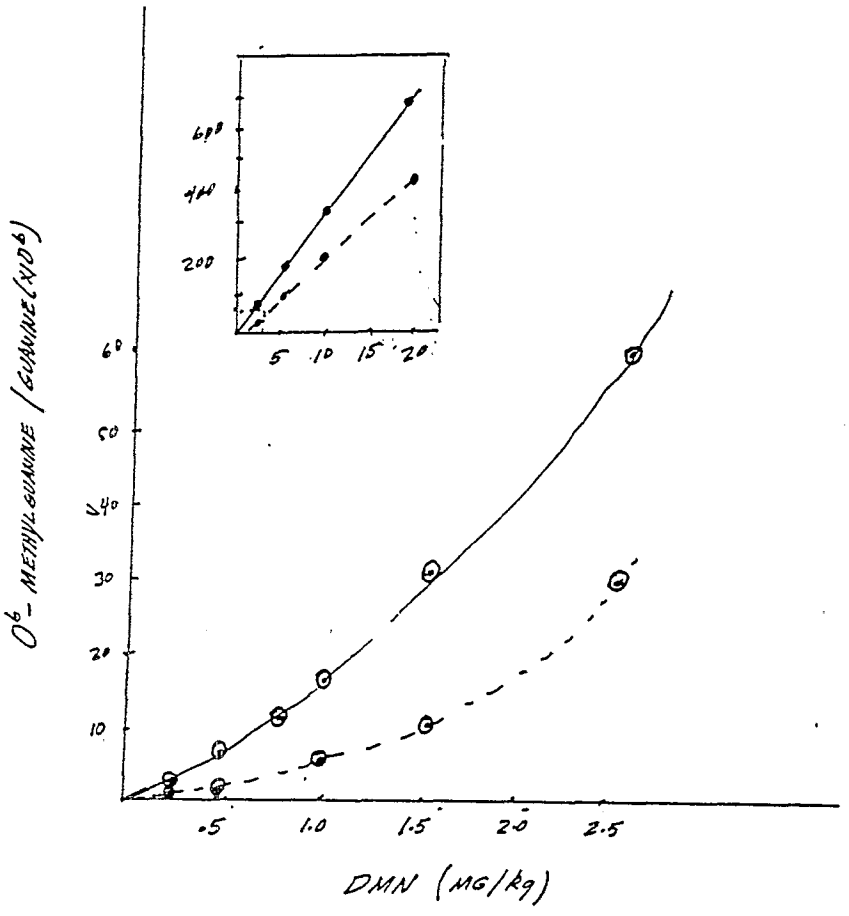
O^6-Methylguanine levels in Rat Liver DNA
After ip administration of single doses of DMN

Pegg (1978)



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Mantel and Byar's Interpretation of Negative Studies

- In a study with 100 animals
- We observe no tumors
- The upper limit on the risk with 99% assurance is: P

$$(1-P)^{100} = (100-99\%) = 0.01$$

$$100 \log(1-P) = \log(0.01)$$

$$1-P = 0.955$$

$$P = 0.045 \text{ or } 4.5\%$$

- Probit corresponding to $0.045 = 3.305$
- Probit corresponding to $10^{-8} = -0.612$
- Upper limit on the risk $= 3.305 - (-0.612)$
  $= 3.917$
- At a slope of one normal deviate per common log it is necessary to reduce the log dose by 3.917 to attain a "safe" level

$$\text{alog}(3.917) = 8,260$$

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

TABLE 4
ESTIMATED VIRTUAL SAFE DOSE (VSD) FOR FOUR MODELS FOR FOURTEEN SUBSTANCES

Estimated VSD at Risk Level 10^{-6}			
One-Hit	Armitage-Doll	Weibull	Multi-Hit
2.0×10^{-5}	1.9×10^{-4}	.52	.80
3.4×10^{-5}	7.9×10^{-4}	4.0×10^{-2}	.28
4.5×10^{-5}	.35	.59	2.3
5.2×10^{-6}	1.6×10^{-3}	1.7×10^{-3}	3.8×10^{-3}
3.2×10^{-5}	1.9×10^{-2}	1.9×10^{-2}	7.7×10^{-2}
2.0×10^{-2}	2.0×10^{-2}	2.1×10^{-9}	3.9×10^{-10}
2.1×10^{-4}	2.2×10^{-4}	2.6×10^{-4}	2.6×10^{-4}
8.4×10^{-8}	4.2×10^{-3}	4.3×10^{-3}	1.3×10^{-2}
1.6×10^{-4}	4.0×10^{-4}	3.1×10^{-2}	3.7×10^{-2}
4.3×10^{-5}	.33	.53	1.1
5.5×10^{-4}	4.5	6.0	33.5
5.7×10^{-6}	2.2×10^{-5}	1.2×10^{-3}	6.7×10^{-3}
2.8×10^{-4}	6.4×10^{-4}	1.7×10^{-2}	4.9×10^{-2}
3.7×10^{-5}	5.7×10^{-5}	1.1×10^{-3}	3.8×10^{-3}

SODIUM SACCHARIN →

From Food Safety Council Report
June 1980 p 149

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

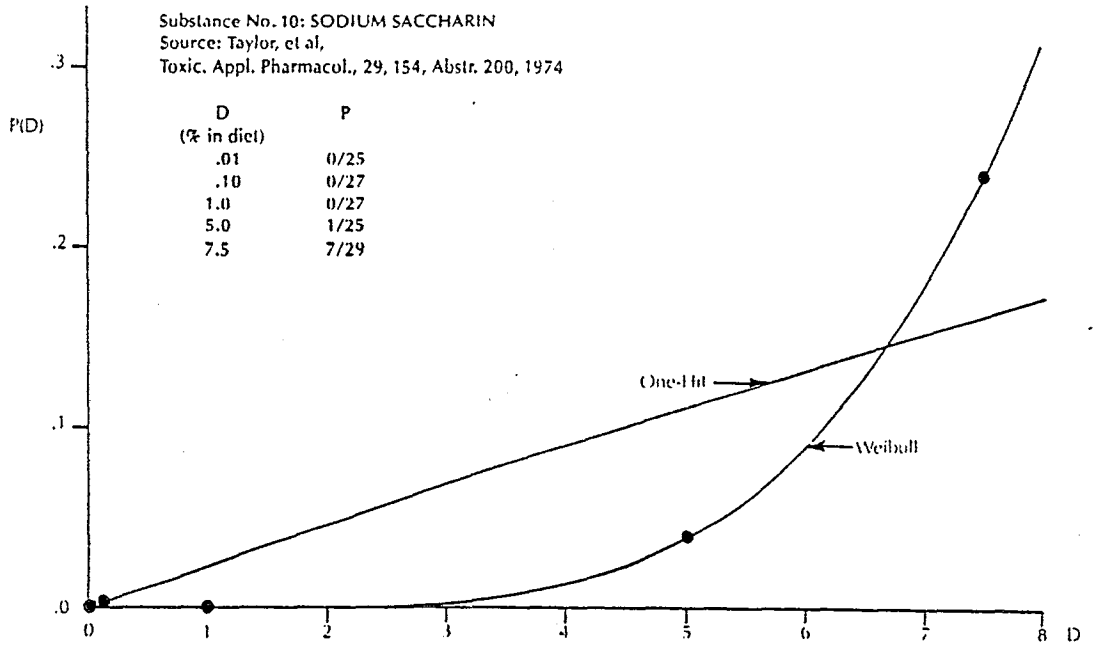

Fig. 2a

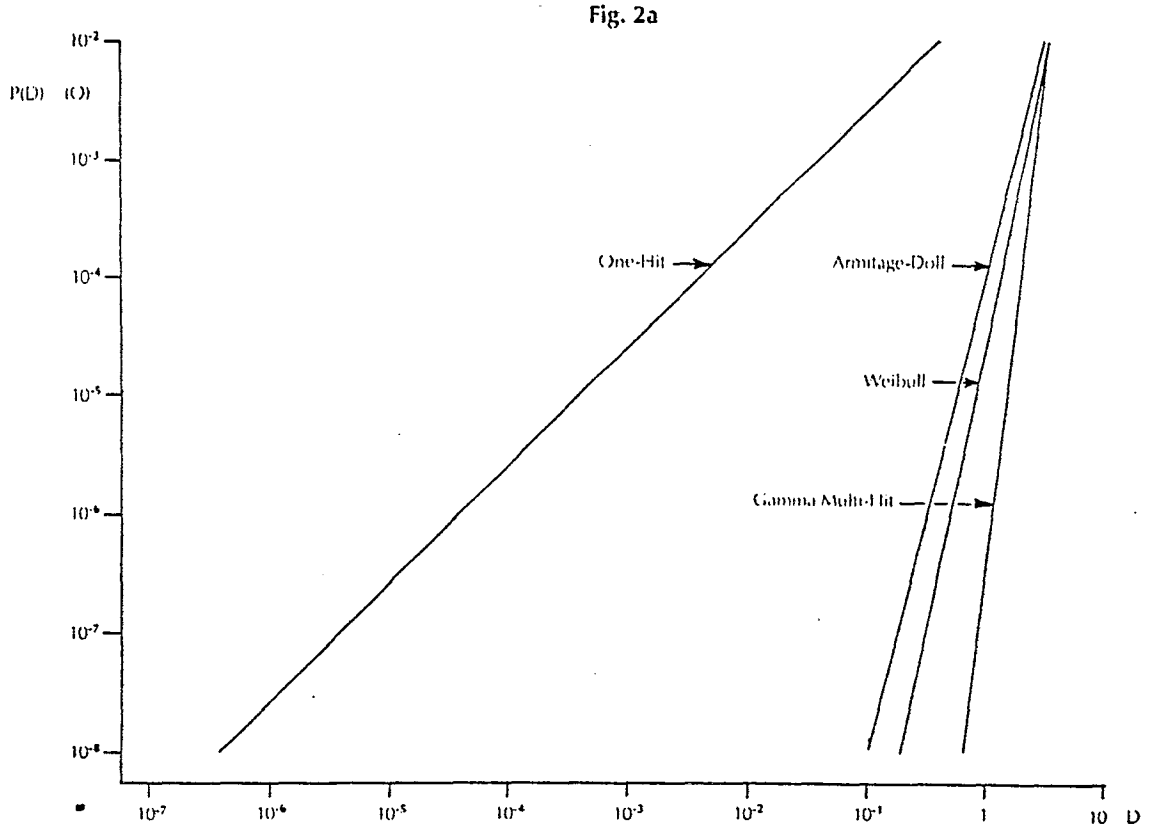

Fig. 2b

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Background Additivity at Low Doses

Crump, Hoel & Peto (1977)

I_1 = Incidence rate due to background carcinogens independent of primary carcinogen

I_2 = Incidence rate due to background carcinogens that act with mechanisms in common with the primary carcinogen

$$I(d) = I_1 + I_2$$

$$= I_1 + F(d + \cdots d^m + \beta d) = I_1 + F(D_0 + \beta d)$$

At low doses:

$$I(d) \cong I_1 + F(D_0) + \beta d \cdot F'(D_0)$$

As long as $F'(D_0)$ is positive, $I(d)$ will be proportional to d for low doses

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

CROUCH, WILSON & BEISE, J. TOXICOL AND ENVIR HEALTH 20:1-10 (1987)

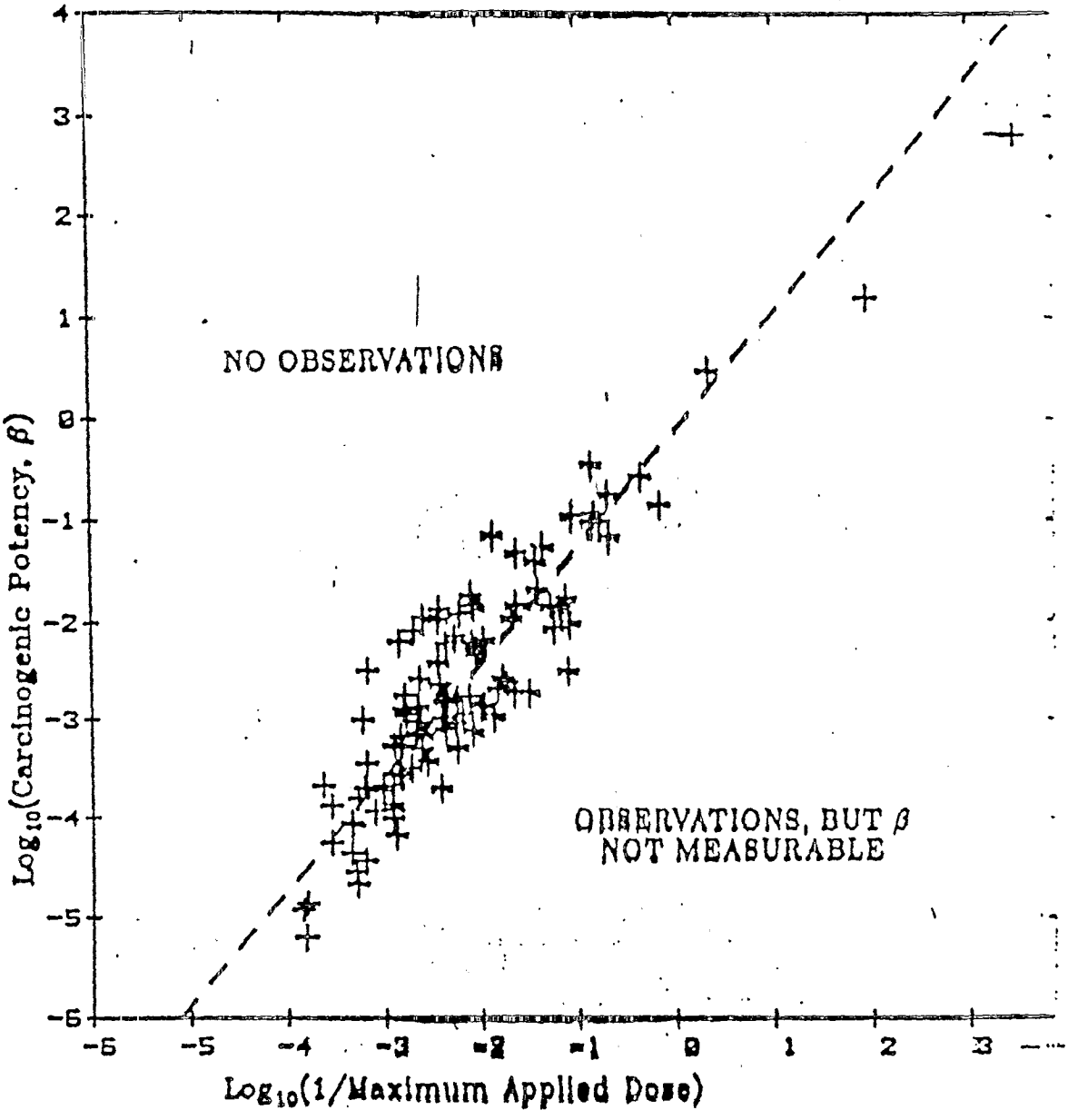

FIGURE 1. Logarithm of carcinogenic potency versus logarithm of the inverse of the [redacted] applied dose for female B6C3F1 mice in the NCI/NTP series of bioassays. Each point repres[redacted] separate experiment. The dotted line is a least-squares fit to those points.

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

BERNSTEIN ET. AL. (1985)

$$MTD_{RATS} = 0.36\ MTD_{MICE}$$

$$R(d) = 1 - e^{-\beta d}$$

$$\beta = -\frac{1}{d}\ln(1-R_d)$$

$$\ln\beta = \ln\left(\frac{1}{d}\right) + \ln\left[-\ln(1-R_D)\right]$$

AT THE TD_{50} ..... $R_D = 1/2$

$$\ln\beta = \ln\left(\frac{1}{TD_{50}}\right) + \ln(\ln 2)$$

$$\beta = \frac{\ln 2}{TD_{50}}$$

RANGE OF TUMOR INCIDENCE .... $10 \leq X \leq 49$

NO ANIMALS 50

BACKGROUND 10%

RANGE OF β ..... $\frac{0.118}{d} \leq \beta \leq \frac{3.807}{d}$

$\beta \Longrightarrow$ 32 FOLD RANGE

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

Reg. Toxicol & Pharmacol 10, 160-73 (1989)

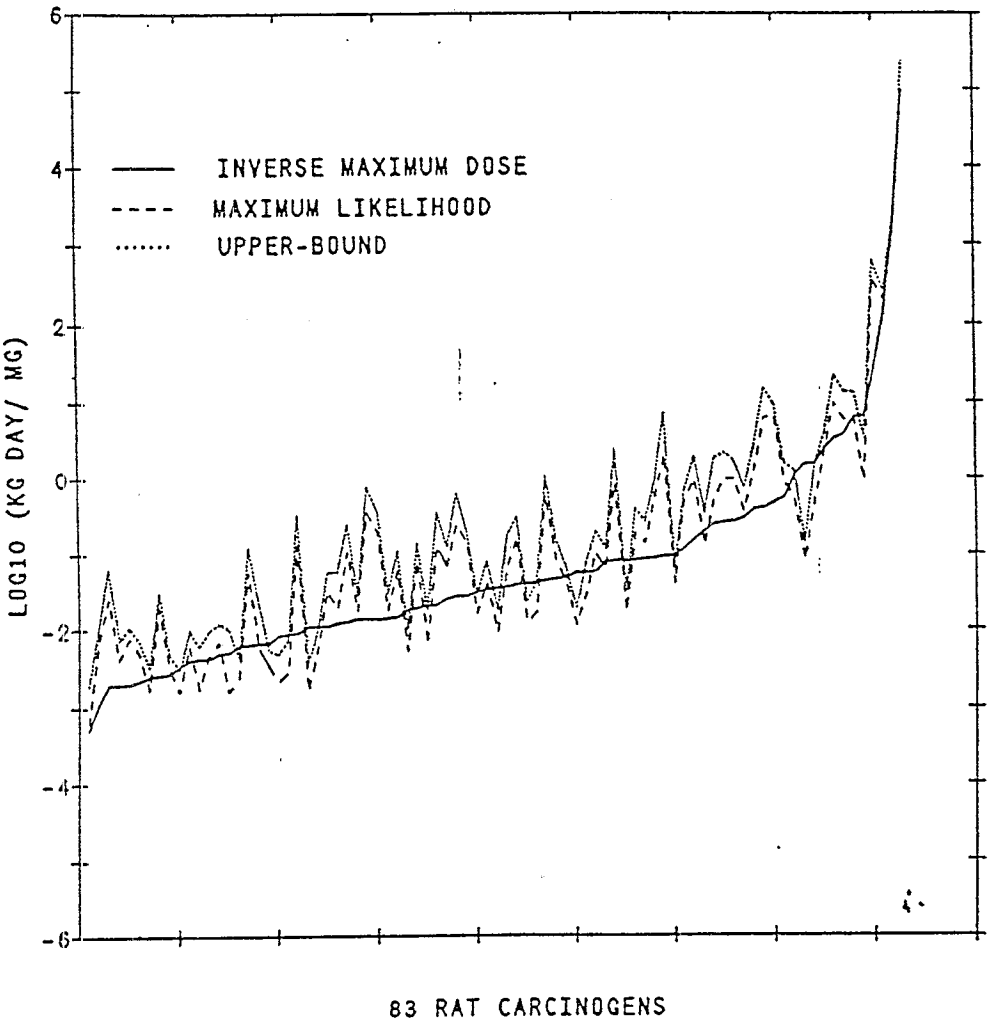

FIG. 4. Comparison of the inverse of the maximum dose tested with maximum likelihood and upper-bound potency estimates of rat carcinogens.

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

DOSE VS CARCINOGENIC ACTION OF DIETHYLNITROSAMINE IN BD II rats.

Druckrey et al, 1963

d daily dosage mg/kg	final Carcinoma yield proportion	D_{50} total dose mg/kg	t_{50} induction time days
14.2	5/5	1000	68
9.6	25/25	963	101
4.8	25/25	660	137
2.4	34/34	460	192
1.2	36/36	285	238
0.6	49/49	213	355
0.3	67/67	137	457
0.15	27/30	91	609
0.075	5/7	6#	840
1:200		1:15	1:12

$$d \cdot t^m = k$$

$$d \cdot t \cdot t^{m-1} = k \qquad dt = \frac{k}{t^{m-1}}$$

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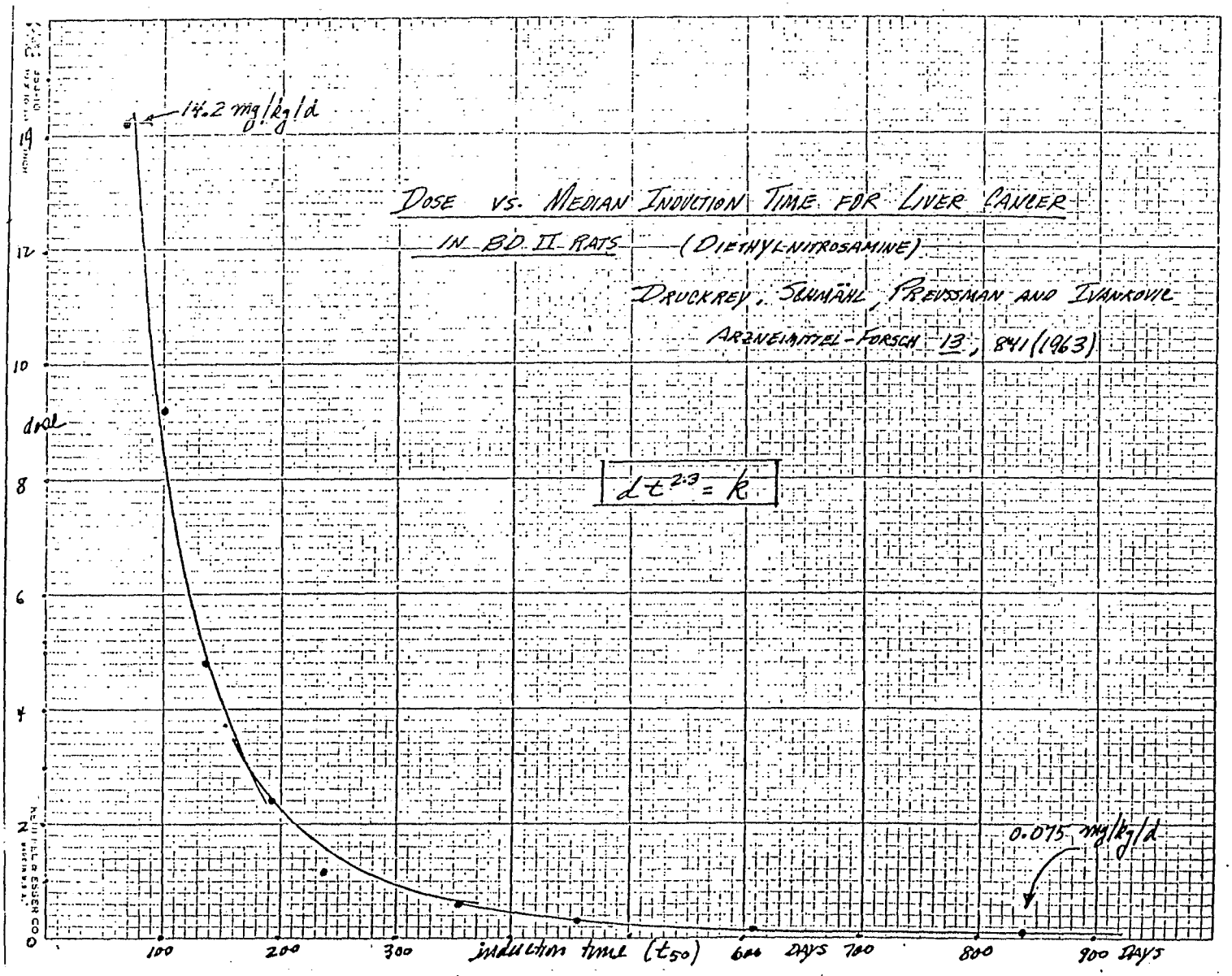
Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000



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Costs of Cancer in the U.S. (1987)

- Deaths — 475,000
- New Cases — 900,000

- Health Care — $ 22 Billion
- Lost Productivity
  - Disability — $ 9
  - Premature Mortality — $ 41

$ 72 Billion

The Surgeon Generals' Report on Nutrition & Health (1988)
DHHS (PHS) Publication No 88-50210

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

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Source: https://www.industrydocuments.ucsf.edu/docs/fzbl0000

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(8) Gehring, P.J., Blau, G.E., Wantanabel, P.G., Pharmacokinetic studies in evaluation of the toxicological and environmental hazard of chemicals. In Adv. in Modern Toxicology - New Concepts in Safety Evaluation, Hemisphere Publishing, Wash., D.C. (1976).

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